

RESEARCH ARTICLE

# Yield Responses of Ruderal Plants to Sucrose in Invasive-Dominated Sagebrush Steppe of the Northern Great Basin

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## Abstract

Restoration of sagebrush-steppe plant communities dominated by the invasive ruderals *Bromus tectorum* (cheatgrass) and *Taeniatherum caput-medusae* (medusahead) can be facilitated by adding carbon (C) to the soil, stimulating microbes to immobilize nitrogen (N) and limit inorganic N availability. Our objectives were to determine responses in (1) cheatgrass and medusahead biomass and seed production; (2) soil microbial biomass C and N; and (3) inorganic soil N to a range of C doses and to calculate the lowest dose that yielded a significant response. In November 2005, we applied 12 C doses ranging from 0 to 2,400 kg C/ha as sucrose to plots sown with cheatgrass and medusahead at two sites in the northern Great Basin. Other ruderal plants established in our plots, and this entire ruderal community was negatively affected by C addition. End-of-year biomass

of the ruderal community decreased approximately by approximately 6% at each site for an increase in C dose of 100 kg C/ha. For the same increase in C, microbial biomass C increased by 2–4 mg/kg in November 2005 and March 2006, but not in July 2006. There was little, if any, microbial soil N uptake, as microbial biomass N increased by 0.3 mg/kg at only one site at the earliest date, in November 2005. Soil nitrate (NO<sub>3</sub><sup>-</sup>) measured via resin capsules placed in situ for the study duration decreased at both sites with increasing C. Although we found no threshold dose of C, for a significant reduction in ruderal biomass, we calculated lowest significant doses of 240–640 kg C/ha.

**Key words:** *Bromus tectorum*, carbon addition, invasive annual grasses, sagebrush steppe, soil nitrogen, *Taeniatherum-caput medusae*.

## Introduction

Human introduction of non-native Eurasian plants, including the annual grasses *Bromus tectorum* (cheatgrass) and *Taeniatherum caput-medusae* (medusahead), to novel environments with Mediterranean-like climates dominated by winter precipitation has fostered epic plant invasions in Australia, South America, and western North America (Novak & Mack 2001). Agriculture and grazing have only recently disturbed the Great Basin in western North America, and soils here are functionally more nutrient-rich than native areas of these ruderal grasses (Blank & Sforza 2007). Annual species like cheatgrass and medusahead are adapted to grow quickly in response to nutrient availability (Grime 1979; Bazzaz 1996), and increased nitrogen (N) availability allows these early-seral

annuals to dominate disturbed sites (McLendon & Redente 1991; Paschke et al. 2000).

These factors have contributed to the replacement of native *Artemisia tridentata* ssp. *wyomingensis* (Wyoming big sagebrush) plant communities in the Great Basin by non-native invasive plant communities, usually dominated by cheatgrass (Knapp 1996) and, more recently, medusahead (Hironaka 1994; Davies & Johnson 2008). Conversion of Wyoming big sagebrush communities to invasive annual grasslands alters ecosystem processes, including inorganic N cycling (Evans et al. 2001; Sperry et al. 2006) wherein nutrient cycling becomes more “leaky” with excess mineral N (Norton et al. 2007). Furthermore, loss of functional diversity in semi-arid bunchgrass communities can increase mineral N availability (Davies et al. 2007). Restoring a conservative N cycle (Norton et al. 2007) and directly limiting soil N (Blumenthal et al. 2003) may be the key to restoring sagebrush steppe and controlling non-native invasives.

Under the current paradigm, carbon (C) addition stimulates C-limited microbes to reproduce or grow and immobilize inorganic soil N. This process limits inorganic N for plant uptake and reduces the competitive advantages of N-demanding invasive annual species over N-conserving late-seral native plants (McLendon & Redente 1992; Zink & Allen 1998; Blumenthal

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et al. 2003). Nutrient limitation via soil C additions should have a greater effect on fast-growing ruderal species than on stress-tolerating perennial grasses, which would benefit from reduced competition from ruderal invasives (Corbin & D'Antonio 2004). Many studies across a variety of ecosystems and plant communities have attempted to manipulate available soil N to decrease non-native plant yields by adding C in various forms (e.g. McLendon & Redente 1992; Young et al. 1998; Reever Morghan & Seastedt 1999; Alpert & Maron 2000; Blumenthal et al. 2003). However, only a handful of studies have directly tested the microbial biomass response to C additions (Jonasson et al. 1996; Zink & Allen 1998; Török et al. 2000; Corbin & D'Antonio 2004), and only two of these studies tested the microbial response to sucrose. Furthermore, plant responses to C addition can be species-specific (Blumenthal et al. 2003; Eschen et al. 2006), indicating that C additions might be effective to limit yields of some invasive species of concern, but not all.

The amount of C applied in previous studies varies widely, but in many experiments was applied in large amounts. In three separate C addition studies in annual-invaded sagebrush communities, application rates of C as sucrose were 1,120 (Young et al. 1998), 1,600 (McLendon & Redente 1992), and 1,740 kg C/ha/year (Young et al. 1999). Adding large quantities of C may be necessary for a specific research question or to offset high site fertility, but high application rates might be needless and costly in N-limited sagebrush-steppe ecosystems, where a lower C dose could be just as effective (Bilbrough & Caldwell 1995; Evans et al. 2001; Blumenthal et al. 2003). Determining the minimum amount of C necessary to control invasives—a threshold dose—could be useful to determine the most cost-efficient amount of C to apply in any given restoration situation.

Our primary research objective was to apply a range of increasing C doses as sucrose to cheatgrass- and medusahead-invaded sites to establish whether and by how much ruderal plant biomass and seed production decreased. We expected to find a threshold dose of C below which lower doses of C would have little or no effect, but above which plant yields would decrease. As C doses increased, an asymptotic threshold would be reached where further additions resulted in no further decline in plant yields. We also hypothesized that dose responses between cheatgrass and medusahead would differ. Finally, to test the mechanism driving the plant responses to C addition, we sought to determine the responses of microbial biomass C and N to soil C, which we predicted would increase, and inorganic soil N to soil C, which we predicted would decrease.

## Methods

### Study Sites

We non-randomly selected two previously established experimental sites, Canyon Creek (Elmore County, Idaho, lat 43°17'37" N, long 115°44'48" W) and Lincoln Bench (Malheur County, Oregon, lat 43°54'25" N, long 117°6'20" W), located approximately 125 km apart on the Snake River Plain

on Bureau of Land Management lands. Both sites formerly supported Wyoming big sagebrush and *Pseudoroegneria spicata* ssp. *spicata* (bluebunch wheatgrass) communities, but both are now dominated by cheatgrass and medusahead. Over time the accumulation of litter and shading from dense cheatgrass and medusahead cover has eliminated the soil crust communities that were historically present on these sites. The climate at both sites is characterized by cool, wet storms in winter and hot, dry summers. Estimated precipitation for the year of this study (September 2005–August 2006) was higher than historical averages at both sites (PRISM Group 2009). Estimated precipitation was 456 and 380 mm at Canyon Creek and Lincoln Bench, respectively, or 129 and 135% of average. Annual species typically germinate after the first available autumn precipitation, and peak growth is from May until June, early in the dry season. Peak growth for native perennial species generally occurs later in the spring and early summer.

Canyon Creek soils were Lanktree or Chilcott loams with fine montmorillonitic, mesic Xerollic Haplargid and Abruptic Xerollic Duragrid (Noe 1991; Bekedam 2004). There are no complete soil surveys for Lincoln Bench, but the site was described as non-sticky silty clay loam to 11 cm in a previous study (Hempy-Mayer & Pyke 2008).

### Experimental Design

We collected cheatgrass and medusahead seeds in June and July 2005 at each site. Seeds were machine-cleaned, and we determined germination rates to be between 70 and 84% (AOSA 2002). We used a randomized complete block design within each site, with three replicates of an 18 × 24-m treatment block established to account for topographic and soil variation within each site. Each block was divided into thirty-six 3 × 4-m plots for a total of 108 plots at each site. Plots consisted of a 2-m<sup>2</sup> sampling area with 1-m buffers. The sampling area was divided further into a 1-m<sup>2</sup> subplot for soil sampling and a 1-m<sup>2</sup> subplot for vegetation sampling. Live plants were killed in each block by applying the herbicide glyphosate at manufacturer's suggested rates in April 2005. We applied additional glyphosate or hand-weeded as necessary later that summer.

We applied experimental treatments from 30 October through 2 November 2005 around the time of the first autumn moisture in hopes of having the strongest effect on annual invasive grass germinants. Limiting available soil N in the autumn and early winter when cheatgrass and medusahead are actively germinating and growing, while native perennial grasses tend to be dormant, should have a disproportionate effect on cheatgrass and medusahead. We first raked plots to scarify the surface and remove remaining vegetation and litter. We then applied treatments in a factorial, random assignment of three species treatments (300 seeds/m<sup>2</sup> of cheatgrass or medusahead, and an unseeded control) crossed with 12 C doses as sucrose (granulated white sugar). Sucrose is approximately 42% C, so we calculated doses equivalent to application rates of 0, 50, 100, 150, 200, 300, 400, 600, 800, 1,200, 1,600, and 2,400 kg C/ha.

First, the appropriate amount of sucrose was mixed with 0.45 kg of sterile, fine sand (at least 75% of particles by volume  $<0.5$  mm) to facilitate even application, and this mixture was hand-broadcasted across the plot and raked lightly. Next, enough seeds by weight for approximately 300 pure live seeds/m<sup>2</sup> of either cheatgrass or medusahead were mixed with 500 mL of rice hulls and hand-broadcasted across each plot. We lightly raked the soil surface again and used a lawn roller to pack down the surface, ensuring good seed-to-soil contact. We then applied a small amount of water with a backpack sprayer to facilitate sucrose adhesion to the topsoil (approximately 1L per 3 × 4-m treatment plot, equal to a 0.08 mm precipitation event). We installed 0.61-m silt fence around each 18 × 24-m block to prevent windblown seeds from moving onto plots and to help keep soil, sucrose, and seeding treatments in place.

### Sample Processing

To determine individual plant biomass and seed production, we randomly selected 16 cheatgrass plants in vegetation subplots planted with cheatgrass, and 16 medusahead plants in subplots planted with medusahead. Cheatgrass ripens earlier than medusahead, so we collected mature cheatgrass plants just prior to seed dispersal on 3–7 and 18–19 June 2006 and returned 4–9 July 2006 to collect medusahead when it matured. Aboveground biomass (referred to hereafter as “biomass”) of each individual was clipped at the soil surface and sealed in a separate paper envelope until seeds could be counted. We counted remaining cheatgrass or medusahead plants in each vegetation subplot, then clipped them at the soil surface and placed them collectively into separate paper bags for each species. We then clipped all other vegetation and composited it together in a paper bag for each subplot. All plant biomass was dried for at least 48 hours at 60°C to a constant mass and weighed to the nearest milligram. Total plant biomass included all oven-dried biomass from each vegetation subplot, including plants collected for individual biomass and seed counts. Cheatgrass and medusahead biomass included all individual plants from each vegetative subplot in which that particular species was planted. Mean relative biomass for cheatgrass, medusahead, and all other species was determined by dividing the biomass of each species by the total biomass of all plants in the plot, and all plots were averaged for each site.

We determined seeds/g for each individual plant by counting the number of mature seeds and dividing that number by the plant's oven-dried biomass, including seed mass. Individual plants were excluded from counts if a majority of seeds were immature or seed heads appeared to have broken off of the plant prior to collection. Seeds/m<sup>2</sup> for cheatgrass and medusahead were calculated by averaging the number of seeds per plant across the 16 individual plants and multiplying by the density of that species in each vegetation subplot.

We collected soil samples for microbial biomass on 18–19 November 2005 to coincide with autumn rains and thus sucrose incorporation into the soil, 4 and 18 March 2006

to coincide with snow melt (later at Lincoln Bench than at Canyon Creek), and 9–10 July 2006 when vegetation was mature. Four 10-cm-deep, 2-cm-diameter soil cores were collected from randomly selected points in each 1-m<sup>2</sup> soil sampling area and composited in sealed polyethylene bags. Samples were kept cool for transport to the lab and were refrigerated for up to 5 days until each sample could be homogenized through a 2-mm mesh sieve before further processing.

We used chloroform fumigation-extraction methods as described in Horwath and Paul (1994) to analyze soil samples for microbial biomass C and N. We modified the method by re-wetting field-dry soils to 60% water holding capacity and incubating in the dark at room temperature for 3 days prior to fumigation and KCl extraction (Horwath & Paul 1994; Saetre & Stark 2005; see Brunson 2008 for more detailed methods). Extracted samples were frozen until analysis on a Shimadzu TOC-V CSH/CSN total organic C analyzer with TNM-1 total N measuring unit (Shimadzu Scientific Instruments, Columbia, MD, U.S.A.). Microbial biomass C and N values were calculated by subtracting the gravimetric soil moisture-corrected post-fumigation values from the corrected pre-fumigation values (Horwath & Paul 1994; Saetre & Stark 2005). We did not use a correction factor because we did not quantify extraction efficiencies.

To estimate plant-available inorganic N over the entire experiment, we buried PST-1 ion-exchange resin capsules (Unibest Inc., Bozeman, MT, U.S.A.) 10-cm deep at three randomly selected locations in each soil subplot. Resins were left in situ from the start of the experiment in November 2005 until experiment end in June or July 2006, then were removed, rinsed with deionized water, and refrigerated until extraction with 60 mL of 2 M KCl for 1 hour on a shaker table. Samples were allowed to settle for 24 hours, then were decanted and filtered through Whatman #42 filters into scintillation vials and frozen until colorimetric analysis (QuickChem Methods 12-107-06-2-A and 12-107-04-1-B) on a Lachat QuickChem8000 autoanalyzer (Lachat Instruments, Milwaukee, Wisconsin). We experienced sample interference problems during colorimetric analysis of ammonium (NH<sub>4</sub><sup>+</sup>-N), so we present only NO<sub>3</sub><sup>-</sup>-N data (Hart & Binkley 1984).

### Statistical Analysis

Seeding treatments were pooled for analysis as there was no significant effect of seeding treatments on relative plant biomass of seeded species at either site ( $F_{[2,298]} = 0$ ,  $p > 0.05$  for Canyon Creek,  $F_{[2,283]} = 0$ ,  $p > 0.05$  for Lincoln Bench). Furthermore, as sites were not randomly selected and seeding success was variable, sites were not considered true replicates and were analyzed separately. Fourteen plots were excluded from analysis because they had been destroyed by loose, blowing silt fence, or were severely eroded.

We used PROC MIXED to perform regressions on total plant biomass, cheatgrass and medusahead biomass, cheatgrass and medusahead seed production, microbial biomass C and N, and soil NO<sub>3</sub><sup>-</sup> (SAS 2002). Data were natural log transformed as necessary to meet model assumptions. Blocks were included

in models as a random effect, so we could not calculate  $r^2$  values to assess model fit. Instead, we compared alternative models using maximum likelihood estimation. The model with the lowest Schwarz Bayesian Information Criterion (BIC) was considered the most parsimonious model. We compared alternative models including the following fixed variables: species seeded, dose, dose<sup>2</sup> (to account for curvature in the data) and their interactions. Dose and dose<sup>2</sup> were treated as continuous variables; all other variables were categorical. The significance of each fixed effect from the most parsimonious model was tested using  $F$ -tests, for which we present estimates and  $p$ -values. If the null model was selected through BIC, we do not present estimates or  $p$ -values (for more detailed model selection criteria and BIC values, see Brunson 2008).

We calculated lowest significant doses for vegetation responses and inorganic soil N by calculating 95% confidence intervals around each regression line and finding the first upper confidence limit that was lower than the mean response of control plots where no C was applied. Using the  $y$ -value from the associated regression point, we solved for the corresponding C dose value  $x$  and called this the lowest significant dose. For microbial biomass C, N, and C:N, which increased in response to C, lowest significant doses were calculated where the 95% lower confidence limit first exceeded the mean response at dose 0 kg C/ha.

## Results

### Plant Biomass and Seed Production

More volunteer plants grew in our treatment plots than expected, and responses of cheatgrass and medusahead were weak in comparison to all ruderal plants in the plots as a whole. Volunteer plants included common ruderal species such as *Lactuca serriola* and *Helianthus annuus* at Canyon Creek and *Sisymbrium altissimum* at Lincoln Bench, and these species accounted for nearly all other plants growing in addition to cheatgrass and medusahead. Mean relative medusahead biomass was 78% at Canyon Creek, and other biomass accounted for 22%. Cheatgrass was the least successful plant at Canyon Creek, accounting for less than 1% of mean relative biomass. At Lincoln Bench, species other than cheatgrass and medusahead dominated the plots, comprising 60% of the mean relative biomass, whereas cheatgrass and medusahead mean relative biomass per plot was 34 and 6%, respectively.

Although seeding treatments were unsuccessful, total plant biomass ( $\text{g/m}^2$ ) of the entire ruderal community in each plot, including cheatgrass and medusahead, decreased exponentially with increasing C at both sites (Fig. 1). For an increase in C of 100 kg C/ha, the back-transformed mean total plant biomass decreased by 6.4% at Canyon Creek and 5.6% at Lincoln Bench ( $F_{[1,98]} = 162.32$ ,  $p < 0.01$ ;  $F_{[1,94]} = 83.57$ ,  $p < 0.01$ , respectively).

Because medusahead established in sufficient quantities at Canyon Creek, we were able to determine that medusahead biomass ( $\text{g/m}^2$ ) at this site decreased with increasing C ( $F_{[1,30]} = 33.81$ ,  $p < 0.01$ , Fig. 2). For an increase in C of

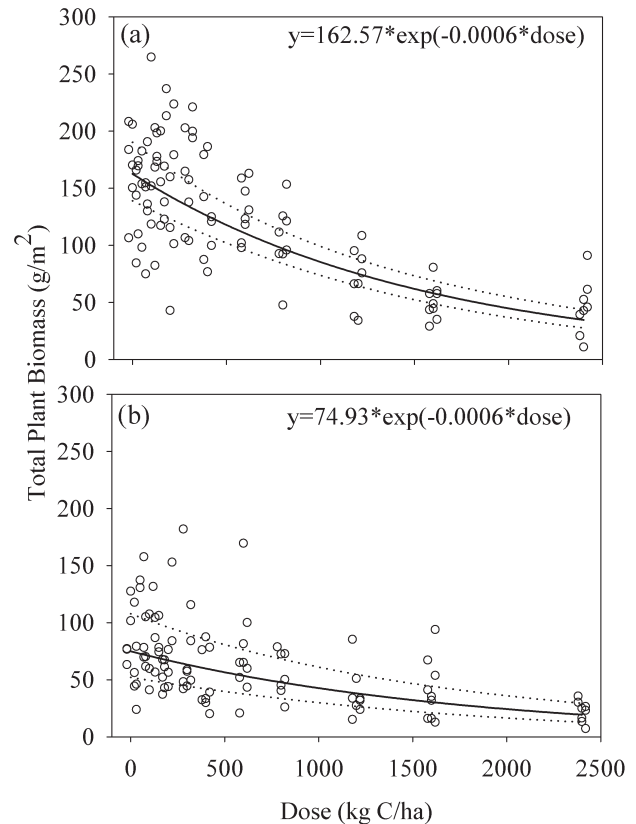


Figure 1. Back-transformed mean total plant biomass ( $\text{g/m}^2$ ) regressed against C dose at (a) Canyon Creek and (b) Lincoln Bench. Solid lines indicate best-fitting regression models and dotted lines indicate 95% confidence intervals. Data points are jittered on the  $x$ -axis for display.

100 kg C/ha, back-transformed mean medusahead biomass decreased by 6.8%.

We were able to detect significant decreases in back-transformed mean medusahead individual biomass ( $\text{g/plant}$ ) at both sites, but the response at Lincoln Bench, where medusahead comprised only 6% of the plant biomass, was smaller than at Canyon Creek (Fig. 3a & 3b). For an increase in C of 100 kg C/ha, medusahead individual biomass decreased by 7.1% at Canyon Creek ( $F_{[1,555]} = 107.09$ ,  $p < 0.01$ ) and 2.7% at Lincoln Bench ( $F_{[1,519]} = 21.77$ ,  $p < 0.01$ ). Cheatgrass individual biomass at Lincoln Bench appeared to decrease when regressed on dose, but this was a weak relationship and the null model had better explanatory power.

Lowest significant C doses for total plant biomass ( $\text{g/m}^2$ ) were 240 kg C/ha at Canyon Creek and 640 kg C/ha at Lincoln Bench. The lowest significant dose for medusahead biomass ( $\text{g/m}^2$ ) was 690 kg C/ha at Canyon Creek, and the lowest significant dose for medusahead individual biomass ( $\text{g/plant}$ ) at Canyon Creek was 300 kg C/ha.

Seed production decreased with increasing C for medusahead only at Canyon Creek (Fig. 3c & 3d). For an increase in C of 100 kg C/ha at Canyon Creek, back-transformed mean medusahead seeds/ $\text{m}^2$  decreased by 6.9% ( $F_{[1,30]} = 23.56$ ,  $p < 0.01$ ) and seeds/g plant decreased by 0.7 seeds

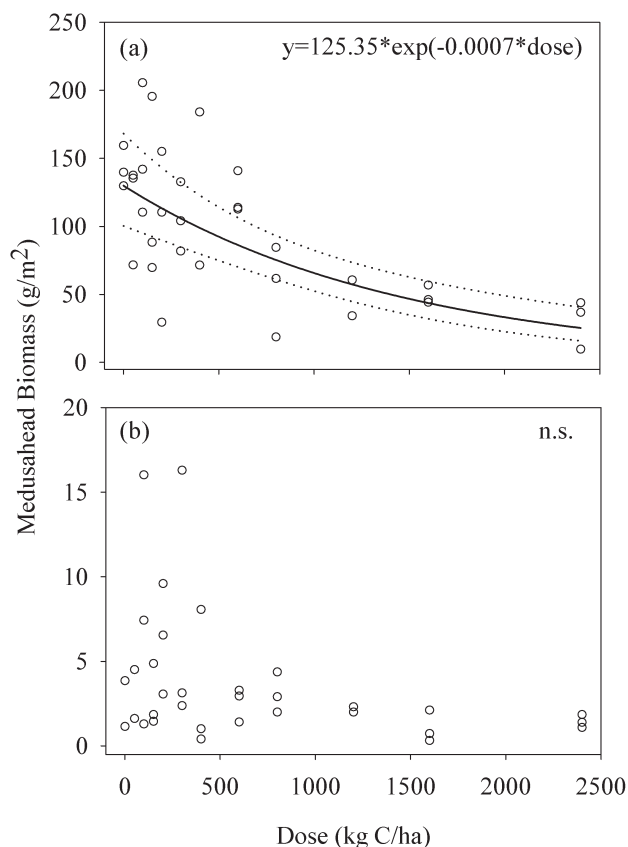


Figure 2. Back-transformed mean medusahead biomass ( $\text{g/m}^2$ ) regressed against C dose at Canyon Creek (a). Raw data with no significant dose effect from Lincoln Bench are shown for comparison (b). The solid line indicates the best-fitting regression model and dotted lines indicate 95% confidence intervals.

( $F_{[1,30]} = 15.89$ ,  $p < 0.01$ ). Lowest significant doses for medusahead seeds/ $\text{m}^2$  and seeds/g plant were 340 and 390 kg C/ha.

#### Microbial Biomass and Inorganic Soil N

Microbial biomass C (mg/kg soil) increased linearly with increasing C dose at both sites in November 2005 and March 2006 (Canyon Creek, November and March:  $F_{[1,56]} = 37.22$ ,  $F_{[1,56]} = 30.85$ ; Lincoln Bench, November and March:  $F_{[1,58]} = 99.95$ ;  $F_{[1,55]} = 33.63$ ; all  $p$ -values  $< 0.01$ ; Fig. 4a & 4b). We present data for November only as March data are similar and July data were not significantly affected by dose (see Brunson 2008 for March and July figures). In November 2005, for an increase in C of 100 kg C/ha, mean microbial biomass C increased by 4.0 mg/kg soil at Canyon Creek and 3.4 mg/kg soil at Lincoln Bench. In March 2006, mean microbial biomass C increased by 2.9 mg/kg soil at Canyon Creek and 2.2 mg/kg soil at Lincoln Bench. Microbial biomass C sampled in July 2006 at both sites was not significantly affected by dose.

Microbial biomass N (mg/kg soil) increased in response to C only at Lincoln Bench in November 2005 ( $F_{[1,58]} =$

19.64,  $p < 0.01$ ; Fig. 4c & 4d). For an increase in C of 100 kg C/ha, microbial biomass N increased by 0.3 mg/kg soil. Microbial biomass C:N increased with C at both sites in November and March, but not in July (Canyon Creek, November and March:  $F_{[1,55]} = 41.42$ ,  $F_{[1,56]} = 73.82$ ; Lincoln Bench, November and March  $F_{[1,58]} = 42.86$ ,  $F_{[1,55]} = 30.58$ ; all  $p$ -values  $< 0.01$ ; data not presented—see Brunson 2008 for figures). For an increase in C of 100 kg C/ha, microbial biomass C:N increased approximately by approximately 0.1 for both sites in November and March.

Ambient site fertility measured as back-transformed mean soil  $\text{NO}_3^-$  extracted from ion-exchange capsules placed in situ from November 2005 through July 2006 was 0.40 mg/g resin (95% CI: 0.19–0.82) and 0.19 mg/g resin (95% CI: 0.09–0.39) at Canyon Creek and Lincoln Bench, respectively. Soil  $\text{NO}_3^-$  (mg/g resin) decreased at both sites with increasing C ( $F_{[1,97]} = 115.88$ ,  $p < 0.01$ ;  $F_{[1,102]} = 77.62$ ,  $p < 0.01$  for Canyon Creek and Lincoln Bench, respectively). For an increase in C of 100 kg C/ha, back-transformed mean soil  $\text{NO}_3^-$  decreased by 15% at Canyon Creek and 9% at Lincoln Bench.

The lowest significant C doses for microbial biomass C ranged from 430 to 560 kg C/ha. For microbial biomass N sampled in November at Lincoln Bench, the lowest significant C dose was 890 kg C/ha. Lowest significant C doses for microbial biomass C:N at Canyon Creek were 350–470 kg C/ha depending on sample date, and 300 and 970 kg C/ha at Lincoln Bench for November and March. Lowest significant C doses for soil  $\text{NO}_3^-$  across both sites were 540–620 kg C/ha.

#### Discussion

We detected a C dose effect on only one of our original species of interest, medusahead, because of the strong response of the entire ruderal community. More notably, the total plant biomass of this community, including cheatgrass and medusahead when present, decreased with increasing C dose, and this response was similar across both sites. Although invasive annual grasses are the greatest impediment to restoration success in this ecosystem (Young & Evans 1973), they respond in consort with other ruderal species to fluctuations in nutrients. This finding is in line with other studies where C addition has been shown to reduce biomass across various ruderal species (Reever Morghan & Seastedt 1999; Blumenthal et al. 2003). Additionally, there was no obvious threshold response to C for total plant biomass or any other variables we measured. We calculated statistically significant minimum doses (lowest significant doses), but we do not know whether these values are biologically significant with respect to reducing the ability of these ruderal species to interfere with native plant establishment in restoration projects.

Blumenthal et al. (2003) found that a minimum C dose of 3,940 kg C/ha decreased weed biomass in tallgrass prairie, but only C applications exceeding 10,000 kg C/ha facilitated native plant growth. They suggested that high site fertility necessitated the large C doses in their study and that sites with lower fertility might not need as much C. We achieved a

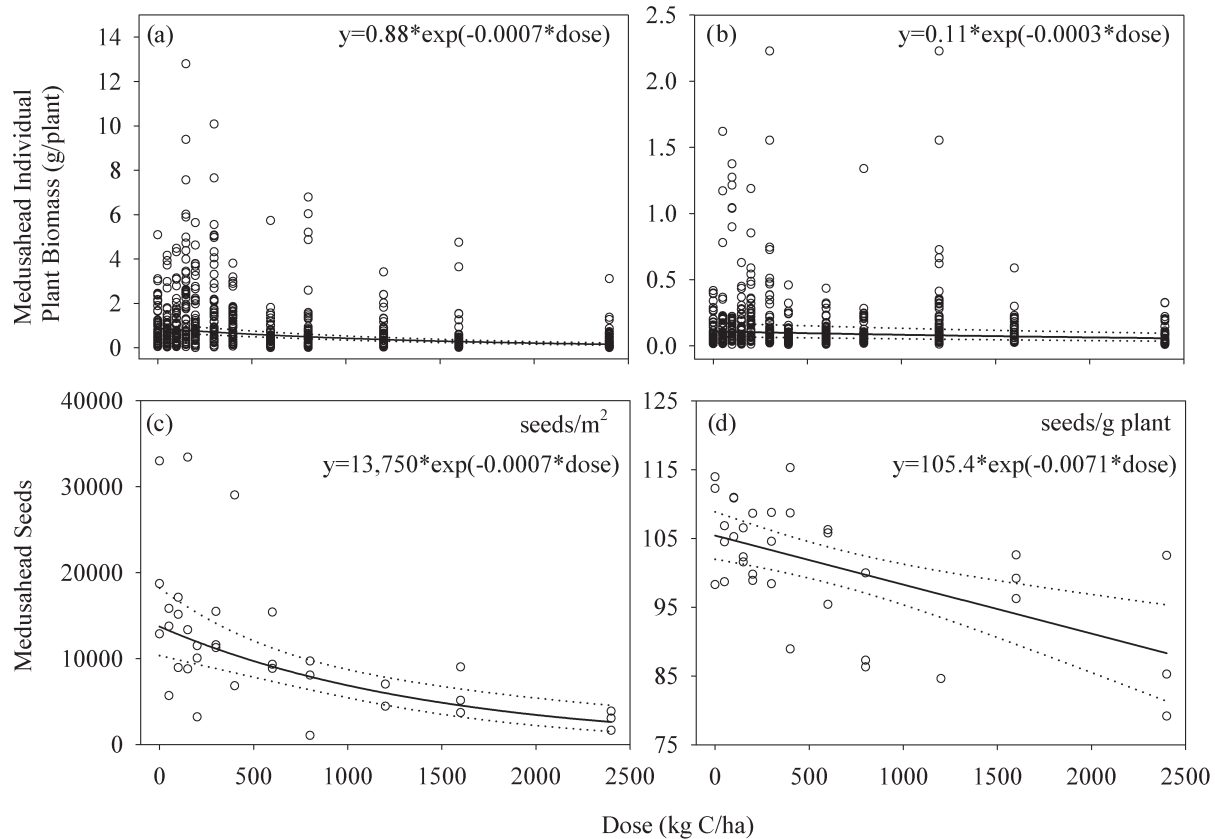


Figure 3. Back-transformed mean medusahead individual plant biomass (g/plant) regressed against C dose at (a) Canyon Creek and (b) Lincoln Bench; (c) back-transformed mean seeds/m<sup>2</sup> and (d) mean seeds/g plant for medusahead at Canyon Creek regressed against C dose. Note different scales on Y axes. Solid lines indicate best-fitting regression models and dotted lines indicate 95% confidence intervals.

significant response with a lower C dose in our experiment in relatively low-nutrient invaded sagebrush steppe, but we only tested the responses of ruderal species to increasing C. Native perennial plant responses to lower C doses should be further studied.

Conversely, Canyon Creek—the site with higher ambient soil NO<sub>3</sub><sup>-</sup> and higher total plant biomass—had a significant ruderal plant biomass decrease (approximately 13%) at a relatively low C dose of 240 kg C/ha. Lincoln Bench total plant biomass decreased much more (approximately 32%) before the difference from controls was significant, and this occurred at a higher dose of 640 kg C/ha. This is a reflection of the asymptotic nature of the total plant biomass response to increasing labile C rather than an indication that sites with higher soil N require less C. Furthermore, at both sites, total plant biomass responded similarly to the same C dose, for example decreasing 25% at 480 kg C/ha, which indicates that initial site fertility (measured as ambient soil NO<sub>3</sub><sup>-</sup> in control plots) might not matter with respect to ruderal plant biomass reduction, at least within the same ecosystem.

Although C additions have shown some promise for reducing invasive plant biomass (Reever Morghan & Seastedt 1999; Alpert & Maron 2000) or benefitting native species (Zink & Allen 1998; Paschke et al. 2000; Blumenthal et al. 2003),

recent studies have shown no lasting effect on establishment of native or perennial species. Corbin and D'Antonio (2004) and Huddleston and Young (2005) found no significant long-term effect of large sawdust amendments (exceeding 3,000 kg C/ha over multi-year studies) on native perennial species. We predicted and observed a negative response of ruderal plant biomass to increasing C over a growing season. Similar to other studies, Mazzola et al. (2008) found that this negative effect is reversed by the next year. This is likely a result of short-lived N immobilization that we observed.

We predicted that adding labile C would increase microbial biomass C most strongly soon after adding it to the soil and that this effect would decrease over time but would still be measureable by experiment's end. We found that C addition did increase microbial biomass C at both sites throughout the winter and early spring (November and March), but this effect did not last through the end of the growing season as there was no dose effect on microbial biomass C in July 2006. Blumenthal et al. (2003) found reductions in soil N over two years in a prairie ecosystem with a one-time C application, but only at high doses using sucrose mixed with sawdust, a more long-lasting source of C. Eschen et al. (2007) observed a decrease in NO<sub>3</sub><sup>-</sup> that persisted for more than a year after C addition as sawdust plus sucrose to fields in the

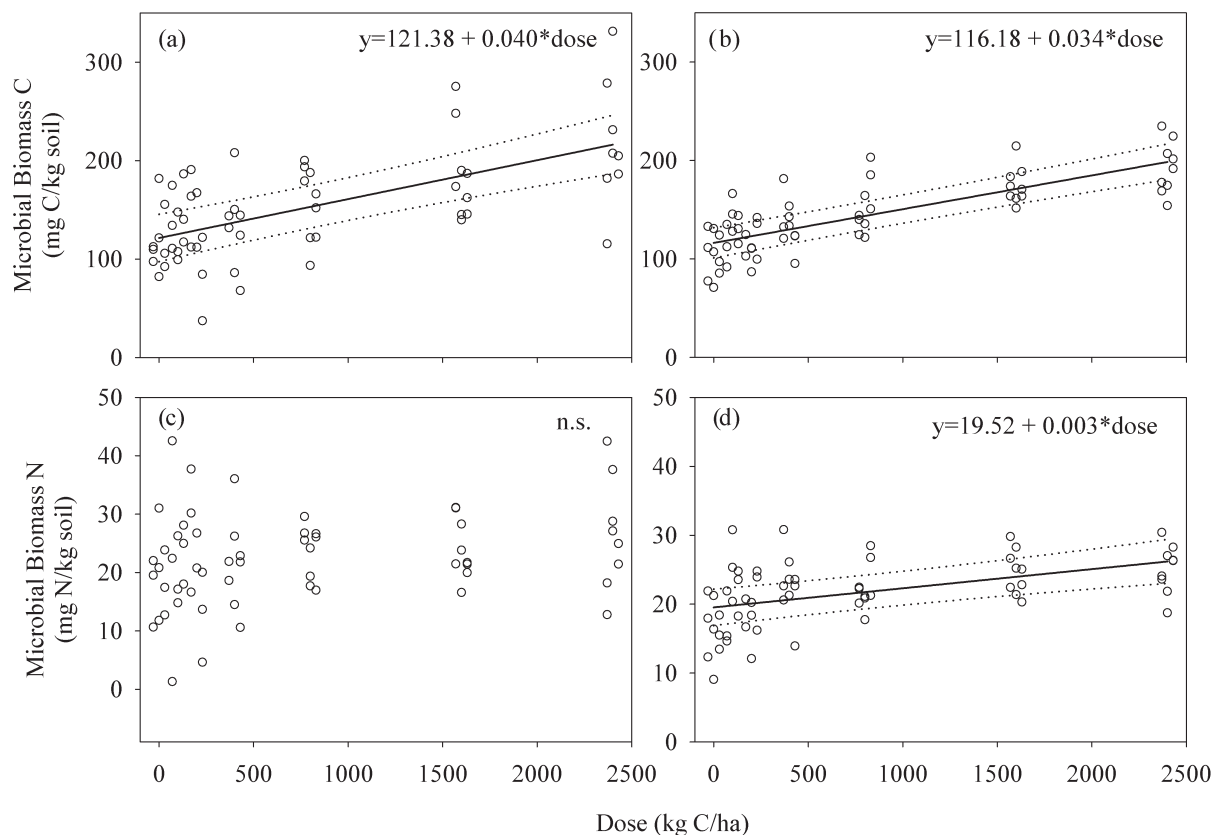


Figure 4. Microbial biomass C (mg/kg soil) in November 2005 regressed against C dose at (a) Canyon Creek and (b) Lincoln Bench, and microbial biomass N (mg/kg soil) in November 2005 regressed against C dose at (c) Canyon Creek and (d) Lincoln Bench. Dotted lines indicate 95% confidence intervals, and data points are jittered on the  $x$ -axis for display.

UK and Switzerland, but the longevity of microbial biomass responses were variable. Although we cannot predict how long soil inorganic N pools would be influenced by a one-time C application, our microbial biomass C results indicate that the microbial community had most likely consumed the entire added C by July and had returned to pre-treatment levels by the end of one growing season.

Surprisingly, changes in microbial biomass N did not parallel increases in microbial biomass C as expected if microbes were actively acquiring and immobilizing inorganic N. Only microbial biomass N samples from November 2005 at Lincoln Bench increased with increasing C dose. Our microbial biomass estimation technique might be insufficient to capture changes in microbial biomass N response. The chloroform fumigation-extraction (CFE) method to estimate microbial biomass has been shown to be less effective in extracting biomass from dry soils compared to wet soils, so we rewetted soils and incubated them prior to fumigation (Sparling & West 1989; Gallardo & Schlesinger 1992; Zagal 1993). This modified-CFE technique produced values for both microbial biomass C and N that are comparable to other studies (e.g. Chen & Stark 2000). Re-wetting cheatgrass soils can release a small, labile N-rich pool of microbial cytoplasm and cells (Chen & Stark 2000) that in our experiment could

have been consumed by the end of the 3-day lab incubation. This may have caused the microbial community to become more N-limited or changed the relative abundance of certain microbial taxa, thus increasing microbial C:N.

Although we found relatively weak changes in microbial biomass N, we did find that soil  $\text{NO}_3^-$  decreased with increasing C dose, which is consistent with other studies in the Great Basin (Witwicki 2005; Mazzola et al. 2008) and elsewhere (Blumenthal et al. 2003; Eschen et al. 2007). Our microbial biomass N samples were instantaneous measurements, whereas soil  $\text{NO}_3^-$ -N captured on resins was measured cumulatively over the entire growing season, perhaps explaining observed differences in these two response variables. Additionally, it is possible that the paradigm of soil N management via microbially mediated N immobilization is incomplete. Future C addition studies should incorporate direct measurements of microbial biomass and employ more appropriate biomass estimation methods for arid soils or characterize microbial community changes in response to C.

## Conclusion

Additions of labile forms of C to semi-arid ecosystems result in reductions to the whole ruderal plant community biomass and

are not limited to invasive annual grasses that are often viewed as the greatest competitors in the system. Given the minimal microbial biomass N response we observed, it is unlikely that a one-time soil C amendment will significantly decrease ruderal plant biomass in the long term. Additionally, microbial biomass N did not mirror the cumulative soil N decrease in response to increasing C, and future studies should consider the possibility that soil N is being lost from the system via other mechanisms, e.g. leaching. Restoration of soil N processes is critical to reestablishing native plant communities in the Great Basin. However, our study suggests that one-time C additions may not be an effective strategy.

### Implications for Practice

- Carbon applied as sucrose can effectively reduce biomass of a mixed community of ruderal plants, but this response will be short-lived and may limit its usefulness as practical restoration tool to a single growing season.
- We found no threshold level of C; that is, no amount of C that we applied resulted in an abrupt change in plant response. For practitioners, the more C added, the greater the reduction in total plant biomass for ruderal plants.
- Carbon applied at amounts lower than 640 kg C/ha may be ineffective in the Great Basin and other semi-arid ecosystems in which Mediterranean annual plants are problematic.

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